Copp. #902

CHRONIC PULMONARY DISEASES

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

RECEIVED JAN 31 1973 COPY

Comm.
Dr. Jacobson
Dr. Loosli
Dr. Sommers

Application for Research Grant
(Use extra pages as needed)

Date: 1/29/73

1. Principal investigator (give title and degrees):

Morton Galdston, M. D., Associate Professor of Medicine

2. Institution & address:

New York University Medical Center
550 First Avenue
New York, N. Y. 10016

3. Department(s) where research will be done or collaboration provided.

Department of Medicine

4. Short title of study:

Protease-Antiprotease Determinants: Role in Etiology and Pathogenesis of Chronic Obstructive Pulmonary Disease

5. Proposed starting date: 7/1/73

6. Estimated time to complete: 3 years

7. Brief description of specific research aims:

Our recent studies suggest the predilection of individuals deficient in alpha$_1$-antitrypsin (A_1AT) to develop chronic obstructive pulmonary disease (COPD) may be partly determined by excess polymorphonuclear leukocyte (PMN) lysosomal neutral protease activity relative to serum antiprotease activity (1,2). These findings and the recent discovery of an antiprotease secreted by ciliated bronchial epithelial cells (3,4) have stimulated us to plan expansion of our investigations of the role of derangements of circulating and local protective mechanisms of lung in the etiology and pathogenesis of COPD, ultimately to devise ways to detect those likely to develop it and methods for prevention and treatment.

Our specific research aims are:

1. to elucidate the role in etiology and pathogenesis of COPD of:
   a. an imbalance between
      (1) serum antiprotease and peripheral leukocyte lysosomal protease activity
      (2) antiprotease and protease activity in bronchial secretions
   b. inflammation in accentuating or inducing these imbalances

2. to investigate the effect of smoking on:
   a. protease-antiprotease balance in peripheral leukocyte lysosomal protease and serum antiprotease activities
   b. protease-antiprotease balance in bronchial secretions
   c. lysosomal protease activity of alveolar macrophages.

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Source: https://www.industrydocuments.ucsf.edu/docs/ypyl0000

8. Brief statement of working hypothesis: 2. 2.

Though individuals with severe A_1AT deficiency (ZZ homozygotes) have a strong predilection to develop COPD at a relatively young age, some do not, even when elderly (5,6). It is controversial whether COPD also occurs with greater frequency among those with moderate A_1AT deficiency, heterozygotes (7,8). Cigarette smoking probably enhances the likelihood in such individuals (9) as it does in those with normal A_1AT (10).

The level of A_1AT may be only one among many determinants of COPD, perhaps genetic, environmental (particularly cigarette smoke) and local protective mechanisms of lung. Whether COPD develops with or without A_1AT deficiency, its rate of progression and pattern of expression could depend upon the interplay and cumulative effects of such factors.

Supportive evidence for the role of lysosomal protease in the etiology and pathogenesis of COPD comes from several experimental observations: human leukocyte lysosomal protease can degrade constituents of lung tissue such as basement membrane (11,12), arterial wall (11,12), collagen (13) and protein polysaccharides of cartilage (14,15); structural changes consistent with human emphysema have been induced in dog lung with aerosolized homogenates of dog and human polymorphonuclear leukocytes and of dog alveolar macrophages of known proteolytic activity (16);

(cont'd pg. 3)

9. Details of experimental design and procedures (append extra pages as necessary)

Subjects

We will investigate the following groups of subjects:

1. those with COPD
   a. homozygous deficient for A_1AT
   b. heterozygous deficient for A_1AT
   c. homozygous for the normal A_1AT gene
2. normals free of lung disease
3. family members of probands deficient in A_1AT
4. individuals undergoing bronchoscopy or with endotracheal or tracheostomy tube in place
5. individuals undergoing lung resection.

The subjects will be volunteers gathered from the following sources:

1. Emphysema Clinic of the Chest Service, Bellevue Hospital, which annually has a census of approximately 200 patients, with about 30 to 40 deaths and an autopsy rate of 25 to 50 per cent, screens about 100 referrals, of which about 50 are added to the Clinic census.
2. patients with COPD admitted to the three hospitals of New York University Medical Center: Bellevue, University and Manhattan Veterans'
3. referrals from attending physicians and from the community
4. family members of those found deficient in A_1AT
5. healthy individuals and patients without lung disease.

We will base the diagnosis of COPD on history, physical findings, chest roentgenogram, electrocardiogram and pulmonary function tests.

Pulmonary function tests will include total lung capacity by the helium dilution method, ratio of residual volume and of functional residual capacity to total lung capacity, arterial pO_2, pCO_2, pH, plasma $BHCO^-_3$ and steady state carbon monoxide diffusing capacity, forced vital capacity (FVC), percentage expired in one second ($FEV_1\%$), forced mid-expiratory flow ($FEF_{25-75\%}$) and forced expiratory flow ($FEF_{200-1200}$).

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Source: https://www.industrydocuments.ucsf.edu/docs/ypyl0000

8. Brief statement of working hypothesis (continued)

purulent sputum contains leukocyte proteases capable of degrading lung tissue; these proteases are inhibited by A_1AT and only to a limited degree by A_1AT-deficient serum (17), which also has a limited capacity to inhibit elastase (18,19) and collagenase (19).

Lung tissue may be particularly vulnerable to such proteases because it has the largest vascular bed of any organ, receives the entire cardiac output, and the potential number of sequestered leukocytes and of alveolar macrophages releasing proteases into the extravascular space is greater than in other organs.

A plausible explanation for the failure of some individuals with A_1AT deficiency to develop COPD might be that they have a concomittant low PMN lysosomal protease activity sufficient to maintain a normal protease-antiprotease balance, whereas those developing lung disease, whether homozygous or heterozygous deficient, or with normal A_1AT, would have normal or increased PMN protease activity and therefore excess protease relative to antiprotease (A_1AT) activity. Such protease-antiprotease imbalance would be greater in homozygotes than in heterozygotes which could account for the greater predilection of the former to develop COPD and at an earlier age (20).

Although there is a history of cigarette smoking in virtually all patients with well-documented COPD (10), only approximately 50% of individuals with a comparable history of long-standing heavy smoking exhibit moderate to advanced emphysema at post mortem (21), again indicating the probable role of several determinants.

The greater predilection of smokers than non-smokers for COPD could be related to a variety of factors tending to induce a less favorable protease-antiprotease balance. Cigarette smokers, particularly those who inhale, have a higher circulating white blood cell count without change in the differential count (22) and therefore are prone to sequester a greater number of PMN in lung. They thus have a potentially richer source for release of lysosomal protease in lung. Also, lung wash of smokers has a greater number of alveolar macrophages, another source for local release of lysosomal protease, and less surfactant (23). The latter suggests one of the local defense mechanisms of lung, surfactant, may be compromised in smokers, since we recently found it to be a potent inhibitor of non-antigenic bacterial cytolytic toxins capable of releasing lysosomal proteases from PMN and alveolar macrophages (24). In addition, pathologic changes in bronchial epithelium of cigarette smokers could alter the local protease-antiprotease balance of lung by inducing enzymes in leukocytes and in alveolar macrophages and by altering the quantity or protective properties of the low molecular weight antiprotease secreted by ciliated bronchial epithelium (3,4) or the rate of diffusion of A_1AT from blood into bronchial secretions (25), without an accompanying change in protease-antiprotease balance in circulating blood.

The low molecular weight antiprotease of bronchial secretions has a similar protease inhibitor spectrum as A_1AT and normally accounts for about 70 per cent of the antiprotease activity of bronchial secretions; the remainder results from the presence of A_1AT (3).

Thus, by investigating protease-antiprotease balance in bronchial secretions along with that in the peripheral blood, under a variety of clinical conditions, we should be able to delineate factors (including cigarette smoking) determining these balances and their role in the etiology and pathogenesis of COPD.

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Source: https://www.industrydocuments.ucsf.edu/docs/ypyl0000

9. Details of experimental design and procedures (continued):

Blood Samples

Fifty cc of peripheral venous blood will be drawn; 2 cc will be used for total blood cell count by a Coulter counter, microhematocrit and hemoglobin determinations, and differential white blood cell count. Eight cc of blood will be used to prepare serum for detecting abnormalities of A_1AT and the remaining 40 cc will be processed to prepare leukocyte lysosomal extract according to Janoff and Scherer (26).

Methods for Detecting Alpha1-Antitrypsin Abnormalities

We will measure serum total trypsin inhibitory capacity (mg trypsin inhibited per ml serum), an index of the total antiprotease activity of serum, according to Eriksson (5). This correlates well with the level of serum A_1AT measured by radial gel immunoassay (27) and by electroimmunodiffusion (20). Values for TIC fall into three groups: normal, 0.80 to 1.40 mg/ml; intermediate low or heterozygous, 0.40 to 0.79 mg/ml; and low or homozygous deficient, less than 0.40 mg/ml (5).

We will perform phenotyping, proteinase inhibitor (Pi), by acid starch gel electrophoresis and crossed electroimmunodiffusion, according to Fagerhol (28). This is a reliable method for detecting heterozygotes with borderline normal TIC and A_1AT, and heterozygotes and homozygotes whose TIC and A_1AT levels rise to normal and low heterozygous range, respectively, with inflammatory disease, estrogens and pregnancy (29,30).

We will use the electroimmunodiffusion method (31,32) to measure A_1AT. We will use a reference serum to be obtained from Dr. Richard C. Talamo, Massachusetts General Hospital, to check on methodology for serum TIC, phenotyping and electroimmunodiffusion and also to standardize a reference serum we will prepare from pooled specimens of normal subjects. Dr. Talamo has kindly consented to assist us in setting up the phenotyping and electroimmunodiffusion methods. Serum specimens will be divided among many small vials and stored at -80 C.

Leukocyte Lysosomal Enzyme Activity

Neutral protease activity of leukocytes has been demonstrated against a broad spectrum of substrates but it is not known how many neutral proteolytic enzymes contribute to tissue damage or to what extent they vary among different individuals. Thus far our most extensive experience has been in measuring two neutral protease activities: elastase-like esterase and leukoprotease. It seems likely that the elastase-like esterase plays an important but not exclusive role in PMN-mediated tissue damage (33). It has recently been found that leukoprotease activity is also partly a measure of elastolytic activity (34). Consistent with this is the highly significant coefficient of correlation, $r = 0.62$ ($P < 0.001$) (80 paired observations), we found between these two measurements (2).

It appears important to investigate whether variation in the level of other neutral leukocyte lysosomal protease activities in addition to those we have been measuring, may, in association with A_1AT, also be determinants of COPD. With this in mind we recently began to measure casein digestion activity.

Other recently reported neutral protease activities of leukocyte lysosomal extract to be considered are: the fibrinogen-splitting enzyme (35); the "labile" protease active on native protein, using the substrate native bovine hemoglobin (17); and the histone-degrading enzyme found in rabbit PMN (36).

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Source: https://www.industrydocuments.ucsf.edu/docs/ypyl0000

9. Details of experimental design and procedures (continued):

Though the role of leukocyte lysosomal acid proteases in tissue damage in vivo is yet to be clearly established it appears that under some conditions neutral and acid protease would act synergistically in extracellular spaces (37). We therefore have also recently begun to measure acid protease activity.

Methods for Measuring Enzyme Activities in Leukocyte Lysosomal Granule Extract:

1. elastase-like esterase with the substrate tertiary-butyloxycarbonyl (t-BOC)-l-alanine p-nitrophenyl ester, according to the method of Visser and Blout (38) as modified by Janoff (39) expressed as the amount of p-nitrophenyl liberated on hydrolysis of the ester $\Delta E_{347.5}/30$ sec/25μg granule extract protein

2. leukoprotease (a guage of total neutral proteolytic activity) with denatured hemoglobin as substrate, according to Anson (40) as modified by Press, Porter and Cebra (41), expressed as TCA supernatants $\Delta E_{280}/10$ min/25μg granule extract protein

3. casein digestion (42) with alpha-casein as substrate, expressed as μg protein digestion/30 min/25μg granule extract protein, as modified by Dr. Aaron Janoff, Department of Pathology, State University of New York at Stony Brook (personal communication)

4. acid protease (pH 3.0) with denatured hemoglobin as substrate by the same method as leukoprotease (40,41) expressed as $\Delta E_{280}/30$ min/50μ granule extract protein

5. beta-glucuronidase, a non-proteolytic leukocyte lysosomal enzyme, serving as a marker enzyme, according to Fishman, using phenolphthalein glucuronic acid as substrate (43), expressed as μg phenolphthalein formed per hour, per mg granule extract protein. Protein will be determined by the Lowry method (44)

Bronchial Secretions

Subjects

Bronchial secretions will be aspirated from healthy part of lung of individuals undergoing bronchoscopic examination, from patients with COPD, and others without lung disease who require an endotracheal or tracheostomy tube.

Annually about 100 patients are examined with the Olympus 5 mm fiberoptic bronchoscope on the Chest Service of Bellevue Hospital; about 250 to 300 undergo bronchoscopic examination on the Surgical and Ear, Nose and Throat Services of the New York University Medical Center; and, in addition, 80 to 90 lung resections are performed. Members of these staffs will collect bronchial secretions for our studies. Dr. Frederick Gorstein, pathologist, will permit us to study resected lung specimens.

We will review the smoking history, clinical course, and pulmonary function tests of those undergoing bronchoscopy or tracheal aspiration.

We will perform the following studies on these secretions:

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1. bacterial culture
2. microscopic examination for purulence
3. protease inhibitory capacity by the total trypsin inhibitory capacity method (5), expressed as mg trypsin inhibited per mg protein in bronchial secretions.

Source: https://www.industrydocuments.ucsf.edu/docs/ypyl0000

9. Details of experimental design and procedures (continued):

This is a measure of the available antiprotease activity in bronchial secretions, i.e., that which has not complexed with protease. Normally, 70 per cent of the antiprotease activity of bronchial secretions results from the low molecular weight antiprotease believed secreted by ciliated bronchial epithelial cells; the remainder is due to A_1AT which diffuses into lung from blood (3).

4. alpha$_1$-antitrypsin, by the electroimmunodiffusion method (31,32) using a modification to be published (see reference 11 in (45), obtained from Dr. Hyslop (personal communication). This will be expressed as A_1AT per mg protein in bronchial secretions.

5. total activity of the low molecular weight antiprotease of bronchial secretions. This is the activity of the free (uncomplexed component) and of the component complexed with protease. The latter is freed when bronchial secretions are processed according to Hochstrasser and co-workers (3,4). Perchloric acid (final concentration 5%) is added to secretions to precipitate higher molecular weight protein components, neutralized with KOH, forming a precipitate of $KClO_4$. Following centrifugation, the supernatant is removed, diluted with borate buffer, pH 8.0, 0.05 M, filtered and concentrated by pressure dialysis with intense stirring. An aliquot is used to measure trypsin inhibitory capacity (5). This will be expressed as mg trypsin inhibited per mg protein in bronchial secretions. Protein will be measured by the Lowry method (44).

6. protease activity, which may be due to lysosomal proteases of alveolar macrophages, leukocytes and bacteria, by the leukoprotease method previously described for leukocytes (40,41) and expressed in appropriate units per mg protein in bronchial secretions (40,41).

7. beta-glucuronidase activity, a measure of lysosomal enzyme release, by the method previously described for leukocytes (43) and expressed in appropriate units per mg protein in bronchial secretions (43).

We will collect a peripheral venous blood sample from the patients whose bronchial secretions are aspirated for studies of protease-antiprotease activity, as previously described, and total and differential white blood cell counts.

Alveolar Macrophages

We will lavage healthy part of resected lung with sterile saline solution according to Cohen and Cline to collect alveolar macrophages (31).

We will wash and resuspend in buffered saline (pH 7.0) the cell button sedimented from aspirated lavage fluid by centrifugation and remove an aliquot for total cell count, differential count using Giemsa stain, observation of cell types by phase-contrast microscopy and for dye-exclusion test with trypan blue (cell viability count).

We will prepare lysates of lysosomal granules of alveolar macrophages according to Janoff (47) and assay them for protease activity using methods already described. We will express enzyme activity per 25μg granule extract protein and also in relation to number of alveolar macrophages per ml.

It is possible that the enzyme activity we find in alveolar macrophages is derived from phagocytized leukocytes destroyed in lung. Studies carried out with Janoff tend to rule this out with regard to elastase-like esterase activity (47); it has yet to be excluded for leukoprotease activity of human alveolar macrophages. Regardless, lysosomal protease of alveolar macrophages would tend to destroy lung tissue in the presence of insufficient local antiprotease capacity.

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Source: https://www.industrydocuments.ucsf.edu/docs/ypyl0000

9. Details of experimental design and procedures (continued):

Analysis of Data

We will have the consultative assistance of an expert statistician, Dr. Agnes Berger, Associate Professor of Biostatistics, School of Public Health of the Faculty of Medicine, Columbia University, in analyzing our results.

We will analyze our data to learn whether:

1. there is a significant correlation among the various neutral protease activities and between each of them and acid protease activity

2. those who have developed COPD have an excess of leukocyte lysosomal protease activity relative to antiprotease activity (low or intermediate low TIC and normal or high protease)

3. those free of lung disease have a normal protease-antiprotease balance (low or intermediate low TIC and corresponding levels of protease activity)

4. those with COPD and the greatest protease-antiprotease imbalance have a more rapid downhill course than those with a lesser imbalance

5. smokers with a protease-antiprotease imbalance have an earlier onset and a more rapid progression of COPD than non-smokers with a comparable imbalance

6. leukocyte lysosomal protease activity is related to the number of mature or immature polymorphonuclear leukocytes

7. there is a population of individuals with COPD with normal TIC and abnormally high leukocyte protease activity

8. there is a pattern of familial transmission of the different leukocyte lysosomal protease activities. Since the enzyme specificity for these activities is not established, definitive conclusions concerning their possible genetic determination will have to await further investigation. Dr. Rody Cox, Section of Genetics, Department of Medicine, New York University Medical Center, will consult in these aspects.

9. the protease-antiprotease balance of bronchial secretions of smokers differs from non-smokers' and, if so, is it related to:

a. diminution of
   (1) total content of low molecular weight antiprotease
   (2) content of A_1AT

b. increase of protease activity from
   (1) increase in number of alveolar macrophages and/or
   (2) enhanced lysosomal protease activity

We will also seek to determine the effect of pulmonary infection and recovery therefrom on protease-antiprotease ballance in bronchial secretions and in peripheral blood; and, similarly, the effect of non-pulmonary infection. Where possible these studies will be carried out in the same individual.

We will also perform serial measurements at intervals of about 6 months to a year in selected patients with COPD with low, intermediate low and normal serum TIC to investigate the relation of clinical progression of the disease with peripheral blood protease-antiprotease balance.

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Source: https://www.industrydocuments.ucsf.edu/docs/ypyl0000

10. Space and facilities available (when elsewhere than item 2 indicates, state location):

Sufficient space is available in the laboratory of the principal investigator, B5, Bellevue Hospital, to carry out all of the planned analytical procedures. In addition, a departmental cold room is available close by.

Among the major items of equipment are: table model high speed and clinical centrifuge, Beckman spectrophotometer, phase contrast microscope, pH meter, Mettler top-load and microbalances, temperature-control water bath, vacuum pump, incubator, sterile hood, flash evaporator, lyophilization apparatus and refrigerator with freeze compartment to -20 C.

Facilities to perform ventilatory pulmonary function measurements are available in the laboratory of the principal investigator. In addition, the facilities and services of the pulmonary function laboratory of the Chest Service, D2, Bellevue Hospital, under the supervision of Dr. Anne L. Davis, collaborator in this proposed research program, are available The major items of equipment here are appropriate spirometers and a helium analyzer to measure total lung volume and subdivisions, dynamic airflow volumes, a blood gas tension analyzer and a pH meter and apparatus to measure carbon monoxide diffusing capacity.

11. Additional facilities required:

None

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12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

Source: https://www.industrydocuments.ucsf.edu/docs/ypyl0000

R: REDACTED MATERIAL

12. Biographical sketches of investigator and other professional personnel:

Horton Galdston, M. D. Role in Proposed Project: Principal Investigator

Title: Associate Professor of Medicine

Birthdate: REDACTED

Place of Birth: REDACTED

Education:

New York University, New York, N.Y., B.S., 1932
Columbia University, New York, N.Y., 1932-1933
New York University College of Medicine, New York, N.Y., M.D., 1937

Honors:

Alpha Omega Alpha
Brooks-Bowen Scholar in Medicine, New York Academy of Medicine
Army Commendation Ribbon for major contributions to lung irritant casualty treatment studies, establishment of objective criteria for evaluation of permanent lung damage by phosgene, development of oxygen therapy equipment
Office of Scientific Research Development certificate for participation in medical research in aviation medicine in World War II.

Research and Professional Experience:

1955-
1968-
1955-
1946-1955
1952-

REDACTED

1944-1946
1943-1944
1942-1957
1942-1943
1941-1942
1939-1941
1937-1939

Major Research Interest: Lung Disease, Pulmonary Physiology, Experimental Pathology

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Source: https://www.industrydocuments.ucsf.edu/docs/ypyl0000

R: REDACTED MATERIAL

12. Biographical sketches of investigator and other professional personnel (continued):

Morton Galdston, M. D. (continued)

Professional Societies:

REDACTED

REDACTED

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12. Biographical sketches of investigator and other professional personnel (continued):

Anne Logan Davis, M. D. Role in Proposed Project: Collaborator

Title: Associate Professor of Clinical Medicine

Birthdate: R

Place of Birth: R

Education:

Wellesley College, B. A., 1945
Columbia University College of Physicians and Surgeons, M. D., 1949
Intern, First (Columbia Univ.) Med. Div., Bellevue Hosp., 1949-1950
Asst. Res. & Res. Phys., Chest Service, Bellevue Hosp., 1954; 1955-1956
Resident Fellow (Amer. Trudeau Soc.), Chest Service, Bellevue Hosp., 1955-1958

Honors:

Alpha Omega Alpha, 1948

Research and Professional Experience:

Hospital

REDACTED	1970-
	1963-1969
	1958-1968
	/1/58-1963
	1954-1955
	1954-1955

University

Assoc. Prof. of Clinical Medicine, NYU School of Med.,	1968-
Assist. Prof. in Med., College of P & S, Columbia Univ.	1964-1968
Assoc. in Med., College of P & S, Columbia Univ.	1960-1964
Instructor in Med., College of P & S, Columbia Univ.	1957-1960

Major Research Interest: Pulmonary Disease

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Source: https://www.industrydocuments.ucsf.edu/docs/ypyl0000

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12. Biographical sketches of investigator and other professional personnel (continued):

Dr. Agnes Berger

Role in Proposed Project: Statistical Consultant (listed in #14 (First Year Budget) under C.)

Biographical Information:

Associate Professor of Biostatistics, School of Public Health of the Faculty of Medicine, Columbia University, New York, N.Y. since 1964.

Ph. D. in Mathemtaics, University of Budapest, 1939.

R

Studied statistics with Naeyman and Wald.

Publications of Medical Interest

Berger, A. and Gold, R.Z., On comparing survival times; Proceedings of the Fourth Berkeley Symposium on Mathematical Statistics and Probability held in 1960; Univ. of Calif. Press.

Gold, R.Z., Berman, S.M., Berger, A., On the question of whether a disease is familial; J. Amer. Statistical Assoc., 62: 409-420, 1967.

Additional Publications have appeared in the following:

Proceedings of the American Mathematical Society
Annals of Mathematical Statistics
Journal of the American Statistical Association

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Source: https://www.industrydocuments.ucsf.edu/docs/ypyl0000

13. Publications of Morton Galdston, M. D. (Principal Investigator):

1. Galdston, M. and Shah, D.O., Surface properties of dipalmitoyl lecithin in relation to lung surfactant, Biochim. et Biophys. Acta, 137: 235, 1967.

2. Galdston, M., Shah, D.O. and Shinowara, G.Y., Isolation and characterization of a lung lipoprotein surfactant, J. Colloid and Interface Sci., 29: 139, 1969.

3. Galdston, M. and Shah, D.O., Lipid-protein association in lung surfactant In Advances in Experimental Medicine and Biology: Surface Chemistry in Biological Systems, Vol. 7, p. 261-274, Plenum Press, New York, 1970.

4. Janoff, A., Rosenberg, R. and Galdston, M., Elastase-like esteroprotease activity in human and rabbit alveolar macrophage granules, Proc. Soc. Exper. Biol. Med., 136: 1054, 1971.

5. Galdston, M., Janoff, A. and Davis, A.L., Familial variation of leucocyte lysosomal protease and alpha$_1$-antitrypsin as determinants in chronic obstructive pulmonary disease. (Accepted for publication in American Review of Respiratory Disease)

In addition, two abstracts dealing with current research activities and referred to in the proposed grant application and submitted for meetings to be held in the spring of 1973 are appended. They are listed as bibliographic references #2 and #24.

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Source: https://www.industrydocuments.ucsf.edu/docs/ypyl0000

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14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Morton Galdston, M. D.	75	-
Anne L. Davis, M. D. (Collaborator)		
Technical		
Christine Gottwalt, Research Technician II	100	R
To be recruited " " "	100	10,261.
Employee Fringe Benefits (14% of salaries)		R
Sub-Total for A		24,358.
B. Consumable supplies (by major categories)		
1. Reagents and Chemicals		1,000.
2. Enzymes		100.
3. Antiserum		500.
4. Glassware		75.
5. Disposable: Syringes and Needles		200.
Lambda and Pasteur Pipettes		125.
Storage Tubes		50.
Sub-Total for B		2,050.
C. Other expenses (itemize)		
1. Statistics: Statistical Consultant (50 hrs. @ $25/hr.)		1,250.
Keypunching and Verification		750.
Computer Usage		300.
2. Maintenance of Equipment		500.
3. Publications, preparation of reports, manuscripts, charts, slides		500.
4. Books, Journals, Photocopying		100.
5. Travel to Meetings		350.
Sub-Total for C		3,750.
Running Total of A + B + C		30,158.
D. Permanent equipment (itemize) *		
1. Electrophoresis Equipment with Attachments		1,750.
2. Amicon Macrosolute Concentrating Apparatus with Accessories		500.
3. Lourdes Model 30-R Refrigerated Centrifuge		2,750.
High Speed Attachment		385.
Large Volume Rotor Head		585.
4. Revco Freeze Chest to -85 C		2,800.
Sub-Total for D		8,810.
E. Indirect costs (15% of A-B-C)		4,524.
Total request		43,492.

*Please see page 15: Justification for Request

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Eq. p.	Indirect Costs	Total
Year 2	26,422.	2,550.	4,250.	0	4,983.	38,205.
Year 3	28,660.	3,050.	4,750.	0	5,469.	41,929.

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Source: https://www.industrydocuments.ucsf.edu/docs/ypyl0000

14 D. Permanent Equipment: Justification for Request

1. Electrophoresis equipment with attachments is essential for phenotyping and measurement of alpha1-antitrypsin content of serum and of bronchial secretions by electroimmunodiffusion. We do not own such equipment.

2. Amicon Macrosolute Concentrating Apparatus with accessories will permit us to separate and concentrate the low molecular weight antiprotease in bronchial secretions.

3. Lourdes Model 30-R refrigerated centrifuge with high speed attachment and large volume rotor head will permit us to handle efficiently the large volumes of lung wash and of blood we will have to process. We selected this model centrifuge because the rotor heads of our table model Lourdes centrifuge fit it. We currently are working under a considerable disadvantage because of the limited capacity of our table model centrifuge and the need to keep it in our refrigerator to attain a reasonably low temperature during centrifugation.

4. Freeze chest to -85 C. It is essential to store leukocyte and alveolar macrophage lysosomal extracts at -80 C to maintain their enzyme activity indefinitely. Facilities for long-term storage are essential for our contemplated research program because of the large number of extracts to be prepared, the practical necessity to analyze at one sitting several specimens prepared over a period of time, the need to maintain composite reference preparations and the desirability of having specimens available to explore new investigative leads. Loss of enzyme activity occurs in about 2 weeks at the lowest temperature attainable, -20 C, in the freeze compartment of our refrigerator.

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Source: https://www.industrydocuments.ucsf.edu/docs/ypyl0000

16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Pulmonary Emphysema: Causes, Prevention and Treatment	Sundry private donors	Approx. $10,000. per yr.	Not applicable

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Protease-antiprotease Determinants: Relation to COPD	N.I.H. HL 14700-01A1 PTHA	$120,620.	7/1/73 - 6/30/76

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal Investigator

Typed Name Morton Goldston, M. D.

Signature Morton Goldston Date 1/29/73

Telephone 212 (Area Code) OREGON 9-3200 (Number) 2681 (Extension)

Checks payable to

New York University Medical Center

Mailing address for checks

550 First Avenue

New York, N. Y. 10016

Responsible officer of institution

Typed Name PETER ARAKELIAN

Title Associate Director

Signature [signature] Date 1/29/73

Telephone 212 (Area Code) OREGON 9-3200 (Number) 3977 (Ext.)

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Bibliographic References

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